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Site-specific recombination in eukaryotic cells mediated by mutant lambda integrases: implications for synaptic complex formation and the reactivity of episomal DNA segments.

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Mutant lambda integrases catalyze site-specific DNA recombination in the absence of accessory factors IHF, XIS, and negative DNA supercoiling. Here

we investigate the effects that a human cellular environment exerts on these reactions in order to (i) gain further insights into mechanistic aspects of recombination in eukaryotic cells and (ii) to further develop the Int system for biotechnological applications. First, we compared intra- and intermolecular integrative as well as excisive recombination pathways on episomal substrates after co-transfection with recombinase expression vectors. Our results demonstrate that, within 24 hours after transfection, intermolecular recombination by mutant integrase is at least as efficient as intramolecular recombination. Second, a significant intermolecular recombination activity was observed between two copies of a recombination site containing only the 21 bp comprising core-type DNA sequence. This basic activity was stimulated several-fold when arm-type DNA sequences were present in addition to core sites. Therefore, one recombination pathway in human cells involves mutant integrases bound solely at core sites, which is reminiscent of the Flp/FRT and Cre/loxP pathways. The stimulatory effect of arm-type sequences could be explained by an increase in integrase concentration in the vicinity of core sites. We show, in addition, that an N-terminal truncated mutant integrase exhibited only a very weak

recombinogenic activity in a eukaryotic background. This result strengthens a functional role for the N-terminal domain in recombination in addition to its arm-type DNA-binding activity. Finally, we demonstrate that low level integrative recombination by wild-type integrase is stimulated when purified integration host factor is co-transfected. This corroborates our previous conclusion that sufficient amounts of eukaryotic protein co-factors, which could functionally replace IHF, are not present in human cells. It also

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provides a potential means to control site-specific recombination in eukaryotic cells. Copyright 2002 Elsevier Science Ltd.

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